# Behavioural activation produced by CRH but not α-helical CRH (CRH-receptor antagonist) when microinfused into the central nucleus of the amygdala under stress-free conditions

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# BEHAVIOURAL ACTIVATION PRODUCED BY CRH BUT NOT α-HELICAL CRH (CRH-RECEPTOR ANTAGONIST) WHEN MICROINFUSED INTO THE CENTRAL NUCLEUS OF THE AMYGDALA UNDER STRESS-FREE CONDITIONS

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## SUMMARY

The central nucleus of the amygdala (CeA) is known to be involved in the regulation of autonomic, neuroendocrine, and behavioural responses in stress situations. The CeA contains large numbers of corticotropin-releasing hormone (CRH)-containing cell bodies and terminals. In the present study we examined (by continuous behaviour observations) the effects of a high dose of CRH (150 ng) and two doses of the CRH-receptor antagonist (α-hCRH: 1.0 and 0.1 μg) after microinfusion into the CeA in freely moving male Wistar rats under stress-free conditions. In comparison with control, α-hCRH infusion did not cause any behavioural activation. In contrast CRH-infusion revealed a long-lasting increase in grooming and exploration with a concomitant decrease in behaviours specified as resting. These results indicate that the CRH system in the CeA does not seem to be activated in stress-free conditions, but its activation is of importance for active behavioural responses.

**Keywords**—Central nucleus of the amygdala (CeA); Stress-free conditions; Corticotropin-releasing hormone (CRH); α-Helical CRH (CRH-receptor antagonist).

## INTRODUCTION

CORTICOTROPIN-RELEASING HORMONE (CRH), a 41-amino-acid peptide, is considered to be a common mediator of stress responses via behavioural, autonomic, and neuroendocrine mechanisms (Cole & Koob, 1991; Davis, 1992; Fisher, 1989). Independent of its adrenocorticotropic properties, numerous studies on the effects of intracerebroventricular (ICV) CRH injections reveal overall increases in blood pressure, heart rate (Brown & Fisher, 1985; Diamant & De Wied, 1991; Fisher et al., 1983; Korte et al., 1993; Kurosawa et al., 1986), plasma catecholamine levels (Bakke et al., 1990; Brown & Fisher, 1985; Korte et al., 1993; Kurosawa et al., 1986), glucose levels (Brown, 1986; Brown et al., 1982), as well as increases in emotionality, fear, and behavioural activity (Cole &

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Koob, 1991; Diamant & De Wied, 1991; Dunn & Berridge, 1990; Koob et al., 1992; Korte et al., 1993; Morley & Levine, 1982; Thatcher–Britton et al., 1986), and decreases in feeding behaviour in different species (Bray, 1992; Dunn & Berridge, 1990; Glowa & Gold, 1991; Gosnell et al., 1983; Morley & Levine, 1982, 1990; Morley et al., 1985). Despite these numerous studies, the exact anatomical sites responsible for the CRH (ICV)-induced actions are less understood.

CRH cell bodies and fibers are located heterogeneously throughout the central nervous system. One of the areas in which a high density of CRH neurons is found, is the central nucleus of the amygdala (CeA), which innervates the periaquaductal gray and the brainstem autonomic nuclei (Danielsen et al., 1989; Gray, 1990a, 1990b; Gray & Magnuson, 1987; Sakanaka et al., 1986).

In a previous study, we have found that local application of a low dose of CRH (30 ng) in the central nucleus of the amygdala (CeA) led to an increase in cardiac response, while this was blocked by the CRH-receptor antagonist, $\alpha$-hCRH (Wiersma et al., 1993). However, infusion of two doses, 0.1 and 1.0 $\mu$g, of $\alpha$-hCRH + CRH (30 ng), resulted in an elevation in plasma corticosterone, an effect which was not observed in CRH treated animals alone. Besides, the CRH-induced behavioural activation was not completely blocked by pretreatment of 0.1 and 1.0 $\mu$g $\alpha$-hCRH. It was suggested that the CRH-antagonist may have possible agonistic properties. Indeed some agonistic properties of $\alpha$-hCRH after ICV administration were already described (Baldwin et al., 1991; Berridge & Dunn, 1987; Winslow et al., 1991).

The present study was designed to substantiate the suggestion that the CRH-antagonist may have possible agonistic properties under stress-free conditions. The aim of this study was to compare the behavioural effects of a high dose of CRH (150 ng) after local infusion into the CeA, to the behavioural responses of the CRH-receptor antagonist after intra-CeA microinfusions.

As some studies indicate that the effects of CRH or its receptor antagonist may depend on the activational state of the CRH system, the experiments were carried out under stress-free resting conditions (Diamant, 1991; Diamant & De Wied, 1991; Krahn et al., 1986). Moreover, several studies suggest that the CRH system is somehow involved in food intake as well (Cole & Koob, 1991; Gosnell et al., 1983; Morley & Levine, 1990). For that reason, the experiments were performed in the presence of fresh food pellets.

## MATERIALS AND METHODS

### *Experimental Animals*

Fifteen male Wistar rats, weighing 290–318 g at the beginning of the experiment, were used. The animals were housed individually in Perspex cages (25 × 25 × 30 cm) and kept in a temperature controlled room (20 ± 2°C) with a 12-h light-dark cycle (lights on from 0830 to 2030h). The experiment was performed during the light period of the cycle (between 0930 and 1430h). Food and water were available ad lib.

### *Surgery*

Each experimental animal was first provided with bilateral permanent stainless-steel brain cannulas (outer diameter 0.3 mm, inner diameter 0.15 mm) for drug infusion, aimed just above the central amygdala (coordinates: 6.7 mm rostral to interaural, lateral 4.0 mm to the midline and ventral 6.2 mm below the dura) according to Paxinos and Watson (1982). The cannulas were implanted stereotaxically and fixed onto the skull by means

of stainless-steel screws and dental cement. The animals were kept under ether anesthesia during the entire surgical procedure.

*Drug Treatment*

Synthetic rat/human CRH (CRF; Sigma Chemical Co., St. Louis, MO) and the CRH receptor antagonist, α-helical CRH9–41 (α-hCRF; Sigma Chemical Co., St. Louis, MO) were dissolved in artificial cerebrospinal fluid (aCSF) with ascorbic acid (100 μg/ml aCSF). CRH was administered in a dose of 150 ng/rat per cannula, α-hCRH at low dose of 0.1 μg (α-hCRH 0.1 μg) and high dose of 1 μg/rat (α-hCRH 1.0 μg) per cannula. The dosages of α-hCRH used are known to antagonise dose-dependently the CRH-induced responses after local application into the CeA in stress-free and stress situations (Wiersma et al., 1993; Wiersma et al., in preparation). The vehicle was sterile artificial cerebrospinal fluid (aCSF) containing 127.64 mM NaCl, 2.55 mM KCl, 1.26 mM $CaCl_2$, and 0.93 mM $MgCl_2 \cdot 6H_2O$. All compounds were infused in a total volume of 1 μl in each cannula during a 7-min period.

*Behavioural Measurements*

Behaviour was recorded on the basis of the following criteria:

- Resting/sleeping: inactive with eyes open or closed.
- Immobility: completely motionless, absence of skeletal and vibrissae movements except those associated with respiration.
- Exploring: investigation of any part of the home cage.
- Grooming: wiping the fur with forepaws and tongue (washing).
- Burying: pushing the bedding material with rapid movements of the snout or forepaws.
- Rearing: sniffing in the air with both forepaws from the floor.
- Eating: chewing food.
- Sniffing: sniffing in the air with paws on the floor.

The various behavioural elements were recorded continuously by means of a keyboard operated microprocessor (EDB, Haren, The Netherlands). The duration and the frequency of these elements were recorded and expressed as the percentage of the total observation period.

*Experimental Procedures*

The experiments were performed in the animals' home cages under stress-free conditions. The rats were trained to habituate to the infusion procedure for at least a few hours during 2 days before the start of the experiments. After at least 10 days recovery from surgery, the rats were tested for the first time. A random treatment design was used in which each rat served as its own control, and received each treatment. Each treatment was separated by a wash-out period of at least 1 week. At $t = -40$ min, the rats were weighed. Subsequently after removal of the cap and obturator, the polyethylene tubing was connected to each cannula. The tubing was filled with the drug infusion solution. The animal was returned to his homecage and the tubing was counterbalanced. At the same time all the food in the cage was removed. At $t = 0$ min, a dish with fresh food pellets was placed in the cage. The microinfusion started then immediately. After termination of the infusion the behavioural observations started. Continuous observations over 63 min were made, subdivided into periods of 7-min each.

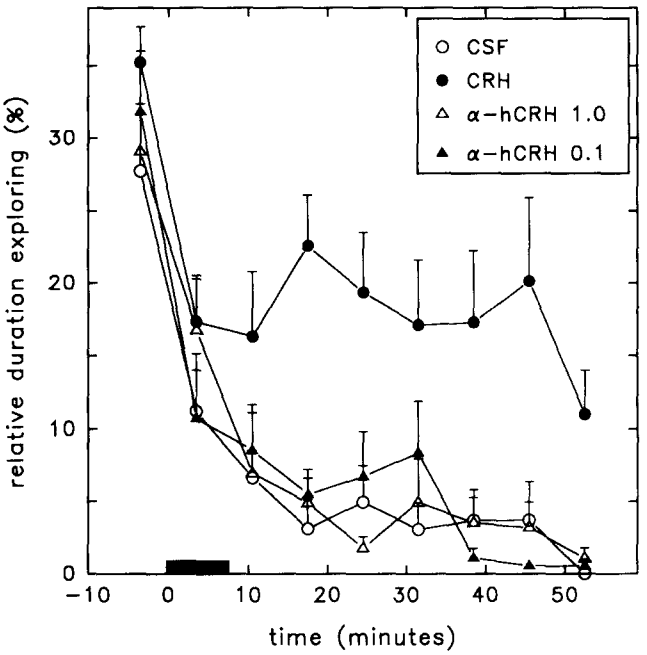


FIG. 1: Duration of exploration (±SEM), during and after microinfusion into the CeA of artificial CSF, 150 ng CRH, or the CRH-receptor antagonist (α-hCRH) in two doses (1.0 and 0.1 μg). At t = 0 min, a plate with fresh food pellets was placed in the cage. The infusion period is indicated by the black horizontal bar.

*Histology*

At the end of the experiments, the rats were deeply anesthetized with sodium pentobarbital (90 mg/kg IP) and perfused intracardially with saline followed by a 4% formaldehyde solution. The brains were postfixed in the same fixative for at least 1 week. Frozen sections of 40 μm were cut and the location of the tip of the cannula was determined on unstained sections.

*Statistical Analysis*

The behavioural data were evaluated using an analysis of variance with repeated measures (ANOVA), followed by the correlated Student's *t*- test. A probability level of $p < .05$ was taken as criterion for significance.

## RESULTS

Histological examination revealed that three of the animals had to be excluded from further analysis because of improper bilateral cannula placement. The cannulae tips had to be localized just above or entering the dorsal edge of the CeA.

The CRH treatment showed pronounced effects on locomotion activity (Fig. 1). The CRH treated animals showed significant more exploring activities compared to the CRH antagonist treated animals and the vehicle group in both the repeated measures ANOVA [ANOVA treatment: CRH-aCSF, $F(1, 22) = 16.12$ $p < .001$; CRH-αhCRH 0.1 μg, $F(1, 22) = 14.06$ $p < .005$; CRH-αhCRH 1.0 μg, $F(1, 22) = 15.83$ $p < .001$] and the Student's

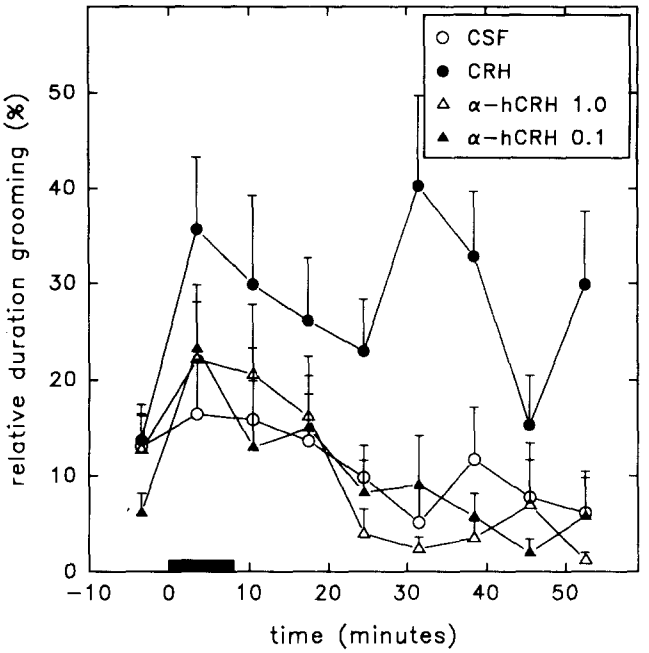


FIG. 2: Duration of grooming (±SEM), during and after microinfusion into the CeA of artificial CSF, 150 ng CRH, or the CRH-receptor antagonist (α-hCRH) in two doses (1.0 and 0.1 μg). At t = 0 min, a plate with fresh food pellets was placed in the cage. The infusion period is indicated by the black horizontal bar.

*t*-test (significance between the groups started at the fourth time interval and lasted till the last measurement).

Also, the immobility response was slightly increased in the CRH-treated animals, but this result was not as clear as the results of exploring and resting responses [treatment: CRH-aCSF, $F(1, 22) = 5.31$ $p < .05$; CRH-αhCRH 0.1 μg, $F(1, 22) = 0.056$ $p = .80$; CRH-αhCRH 1.0 μg, $F(1, 22) = 8.30$ $p < .01$].

The CRH treatment also caused a significant increase in grooming (Fig. 2) compared to the three other groups during the whole 63-min observation period [ANOVA treatment: CRH-aCSF, $F(1, 22) = 8.20$ $p < .01$; CRH-αhCRH 0.1 μg, $F(1, 22) = 15.09$ $p < .005$; CRH-αhCRH 1.0 μg, $F(1, 22) = 13.629$ $p < .005$].

The infusion of aCSF, and both concentrations of the CRH-receptor antagonist resulted in a steady increase in resting behaviour to 90% of the total time observed at the end of the observation period (Fig. 3). However, the CRH infusion caused only a small increase in resting behaviour, which resulted in a maximum of 40% of the total time spent resting at the last time interval (Fig. 1). Comparing the CRH with all the other treatments analysis of variance showed highly significant treatment effects [CRH-aCSF, $F(1, 22) = 15.68$ $p < .001$; CRH-αhCRH 0.1 μg, $F(1, 22) = 14.44$ $p < .005$; CRH-αhCRH 1.0 μg, $F(1, 22) = 24.18$ $p < .0005$]. Also significant differences between time and group × time interactions were found. The Student's *t*-test revealed that the significant decreases in the CRH compared with both of the CRH-receptor antagonist treatments started at the third observation time interval, whereas the significant differences between the vehicle and the CRH treatment started at the second time interval and lasted until the end of the total observation period.

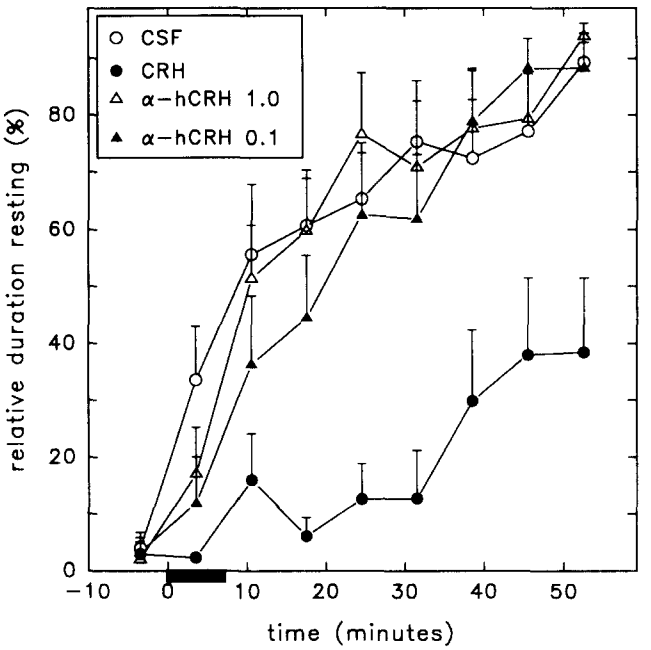


FIG. 3: Duration of resting (±SEM), during and after microinfusion into the CeA of artificial CSF, 150 ng CRH, or the CRH-receptor antagonist (α-hCRH) in two doses (1.0 and 0.1 μg). At t = 0 min, a plate with fresh food pellets was placed in the cage. The infusion period is indicated by the black horizontal bar.

No differences were found between the three treatment groups and the vehicle group in the other observed behaviours.

## DISCUSSION

The main findings of the present experiment were that local application of a high dose of CRH into the CeA resulted in a long-lasting increase in exploration and grooming behaviour, with a concomitant decrease in time spent resting during the entire observation period. Administration of the CRH-receptor antagonist in two concentrations in the CeA did not cause discernible effects on behaviour in rats under stress-free conditions.

The increase in behavioural activity, especially locomotor and grooming activities, as a result of the CRH infusion in the CeA in the rats' home cage, is consistent with observations that CRH administered both ICV (Diamant & De Wied, 1991; Eaves et al., 1985; Sutton et al., 1982) and locally into the CeA produces behavioural activation in the rat (Lee & Sung, 1989; Lee & Tsai, 1989; Wiersma et al., 1993). However, CRH infusion in the CeA, in a dose five times lower as the dose used in the present study, did not result in an excessive grooming response (Wiersma et al., 1993). This suggest that CRH administration at high doses in the CeA induces a nonspecific behavioural response. This assumption is in accordance with the results of ICV CRH applications (Britton et al., 1982; Diamant & De Wied, 1991; Morley & Levine, 1982; Sherman & Kalin, 1988; Spruijt et al., 1992). It was suggested that the threshold for CRH to induce grooming may depend on the testing conditions (Diamant & De Wied, 1991; Dunn &

Berridge, 1990). At low doses, CRH was found sufficient to induce grooming when rats were treated in a shock chamber, whereas higher doses were necessary to produce a similar effect when rats were tested in an open field or the home cage (Diamant & De Wied, 1991; Morley & Levine, 1982; Thatcher–Britton et al., 1986; Veldhuis & De Wied, 1984). Excessive grooming may represent a displacement activity during an increased arousal state and it probably serves as restoration of the homeostatic status of the rat (Cohen & Price, 1979; Jolles et al., 1979; Sherman & Kalin, 1988; Spruijt et al., 1992).

The finding that $\alpha$-hCRH in the CeA fails to affect behavioural activation in the stress-free condition supports the results of different reports injecting CRH and the antagonist in the ventricles (ICV), suggesting that while the CRH-antagonist blocked CRH-induced and stress-induced effects (Baldwin et al., 1991; Berridge & Dunn, 1987; Cole et al., 1992; Heinrichs et al., 1992; Tazi et al., 1987), the CRH antagonist had no agonistic effect of its own (Cole & Koob, 1991; Heinrichs et al., 1992; Krahn et al., 1986; Thatcher–Britton et al., 1986). For example, Krahn and colleagues (1986) found that after ICV $\alpha$-hCRH administration, the CRH antagonist did not change food intake in fasted but otherwise unstressed rats. However, this finding stands in contrast to the increase in feeding with ICV administration of $\alpha$-hCRH seen after CRH treatment (Krahn et al., 1986). They suggest that the direction of feeding effects of $\alpha$-hCRH is dependent on the state of the CRH system in the subject tested. Furthermore, in a recent study, intra-amygdaloid administration of the CRH-antagonist dose-dependently reversed the effect of exposure to an aggressive resident, without altering the behaviour of unstressed rats (Heinrichs et al., 1992). The results of the present and previous studies (Wiersma et al., 1993), infusing the CRH-receptor antagonist into the CeA under stress-free and CRH-activated conditions, are in agreement with the results of Krahn and collegues (1986) after ICV and Heinrichs and co-workers (1992) after intra-amygdaloid administration. This suggests that the CeA may be the possible anatomical localization which can be responsible for some of the ICV CRH-induced and ICV $\alpha$-hCRH-induced behavioural responses.

In the present study no effects of either $\alpha$-hCRH or CRH treatment on feeding behaviour was observed. CRH given ICV is known to influence metabolism by eliciting an increase in plasma concentration of glucagon and glucose and a decrease in plasma insulin levels (Brown, 1986; Brown et al., 1982), which in turn results in a decrease in feeding behaviour (Bray, 1992; Dunn & Berridge, 1990; Glowa & Gold, 1991; Gosnell et al., 1983; Levine et al., 1983; Morley & Levine, 1982, 1990; Morley et al., 1985). On the other hand, ICV injection of the CRH receptor antagonist partially reversed the CRH- or stress-induced anorexic effects. It seems that the paraventricular nucleus of the hypothalamus (PVN) is the site within the central nervous system at which CRH produces its inhibitory effect on feeding, as studies with intrahypothalamic infusions revealed that only CRH in the PVN dose-dependently increased serum glucose levels (Cole & Koob, 1991; Gunion et al., 1988; Morley & Levine, 1990). As no direct CRH projections from the CeA to the PVN are known (Gray, 1990b) and no effect on feeding behaviour after either CRH or CRH-antagonist infusion in the CeA occurred (Wiersma et al., 1993), it appears that the CeA is probably not involved in the ICV CRH-induced decrease in feeding response.

In conclusion, our results and earlier work done in this laboratory reveal that the CeA may be responsible for some of the ICV CRH-induced behavioural activation, such as the dose-dependent increase in grooming, and the activation in exploring responses. Further, the present study shows that the $\alpha$-hCRH infusion in the CeA under stress-

free conditions does not have any behavioural effects. Probably, the possible agonistic properties of $\alpha$-hCRH depends on the activational state of the total CRH system in the CNS. In the present experiment we assumed that the CRH system was not activated as a result of the stress-free conditions. It is known that the CRH system in the CNS will react differently to the same stimuli if the CRH system is activated, in stress situations, or not, in stress-free situations. The present results are in accordance with the idea that the CRH system in the CeA is not activated in stress-free conditions.

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